UCRL-92834
PREPRINT

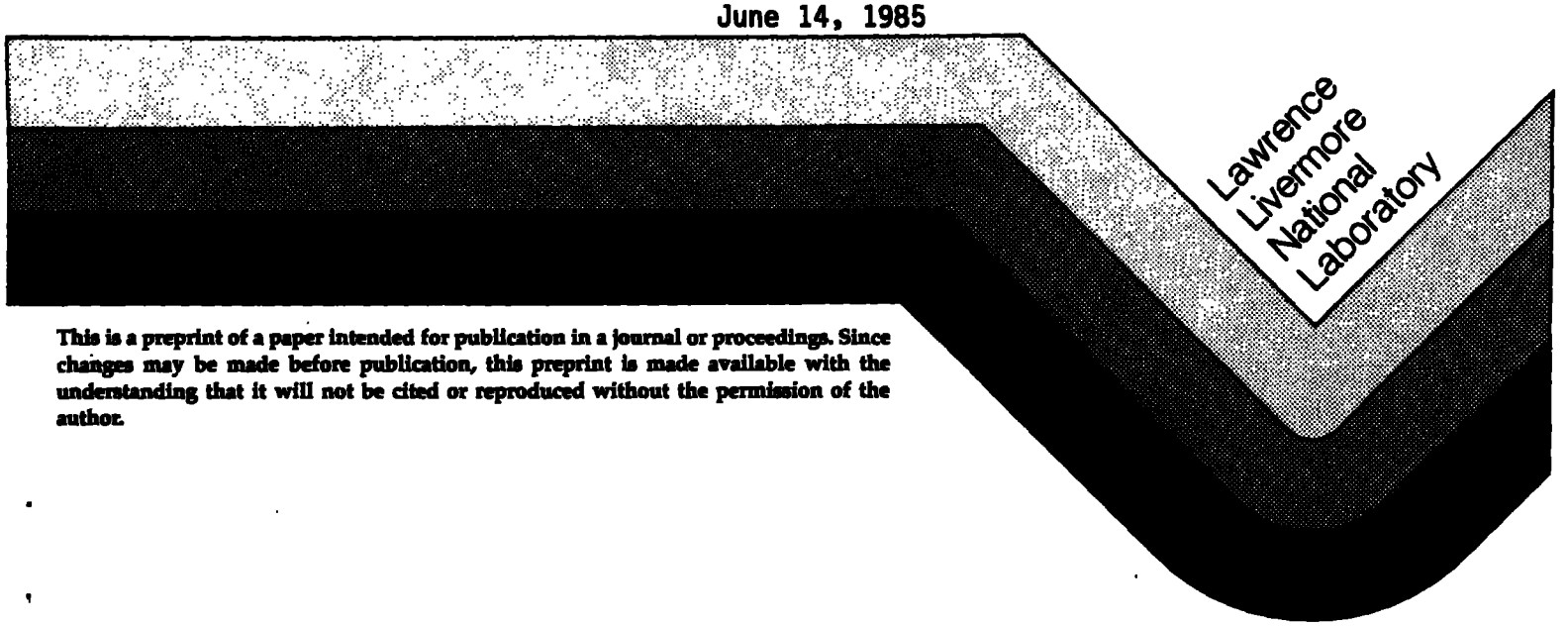
A SOMATIC CELL AND MOLECULAR GENETICS APPROACH TO DNA REPAIR AND MUTAGENESIS

Larry H. Thompson

BIOMEDICAL SCIENCES DIVISION

This paper was prepared for submittal to:
"Fourth International Conference on
Environmental Mutagens"
Stockholm, Sweden, June 24-28, 1985

June 14, 1985



DISCLAIMER

A SOMATIC CELL AND MOLECULAR GENETICS APPROACH TO DNA REPAIR AND MUTAGENESIS

Larry H. Thompson

Biomedical Sciences Division, Lawrence Livermore National Laboratory, P.O. Box 5507, Livermore, California, 94550, U.S.A.

INTRODUCTION

Our laboratory (Thompson et al. 1980) and others (Stamato and Waldren, 1977; Sato and Hieda, 1979; Busch et al. 1980) showed the feasibility of isolating mutant cell lines induced in culture that had markedly increased sensitivity to far ultraviolet (UV) radiation or certain chemical mutagens. In the CHO cell line, UV-sensitive mutants representing five genetic complementation groups have been identified (Thompson et al., 1981; Thompson and Carrano, 1983). Mutants from each of these groups were shown to be defective in performing the incision step of repair after exposure to UV (Thompson et al. 1982a). In this respect, they closely resemble mutant human cells from individuals with the cancer-prone xeroderma pigmentosum (XP) syndrome (Cleaver, 1983). Whether any of the rodent cell mutants are altered in the same genes as XP cells remains to be determined (Thompson et al., 1985a).

One of the most exciting aspects of the hamster and mouse repair mutants is the finding that the defects can be genetically complemented by human genes. This relationship has been shown using both DNA-mediated gene transfer (Rubin et al., 1983) and cell fusion to produce hybrid cells (Hori et al., 1983; Thompson et al., 1985b). Recently the first molecular cloning of a human repair gene associated with the repair of damage from UV radiation and mitomycin C was accomplished by Westerveld and coworkers (1984). Thus, it now seems possible to identify and isolate many human genes involved in repair by their ability to complement mutations

present in rodent cell lines. This somatic cell genetics approach to determining human repair genes may be limited only by the diversity of mutants that can be obtained from a variety of established cell lines, including human lines. Thus, a detailed molecular dissection of repair pathways may eventually become possible, as in the case of lower eukaryotes and prokaryotes (Walker et al., 1985).

Repair-deficient cell lines are valuable for mutagenesis testing because of their hypersensitivity to induced mutation and induced sister-chromatid exchange, as well as to cell killing (Thompson et al., 1982a, 1982b, 1982c, 1983a). In one instance of a bacterial mutagen that is known to be present in cooked meats, the extra sensitivity of excision-repair-deficient cells was helpful in establishing the ability of the compound to produce genetic damage in CHO cells (Thompson et al., 1983b). It has also been possible to use the repair mutants to devise a simple, rapid assay for detecting DNA-damaging agents based on differential cytotoxicity between normal cells and repair-deficient strains (Hoy et al., 1984, 1985).

Repair-deficient lines also have application to issues such as the role of repair in cell cycle variations in response to mutagens (Wood and Burki, 1982; Thompson et al., 1984). These studies have indicated that nucleotide excision repair is responsible for much of the cyclic change in sensitivity to killing that is seen with agents producing bulky lesions.

RESULTS

Two Phenotypes of Nucleotide Excision Repair Mutants of CHO Cells

The UV-sensitive CHO mutants belonging to 5 complementation groups were found to be defective in nucleotide excision repair on the basis of their inability to perform the incision step after UV exposure (Thompson et al., 1982a) and to remove the bulky adducts of 7-bromomethylbenz[a]anthracene (Thompson et al., 1984). Although mutants from the different complementation groups have similar sensitivity to killing by UV radiation or monofunctional mutagens forming bulky adducts (Thompson et al., 1981; Hoy et al., 1984), the sensitivity to

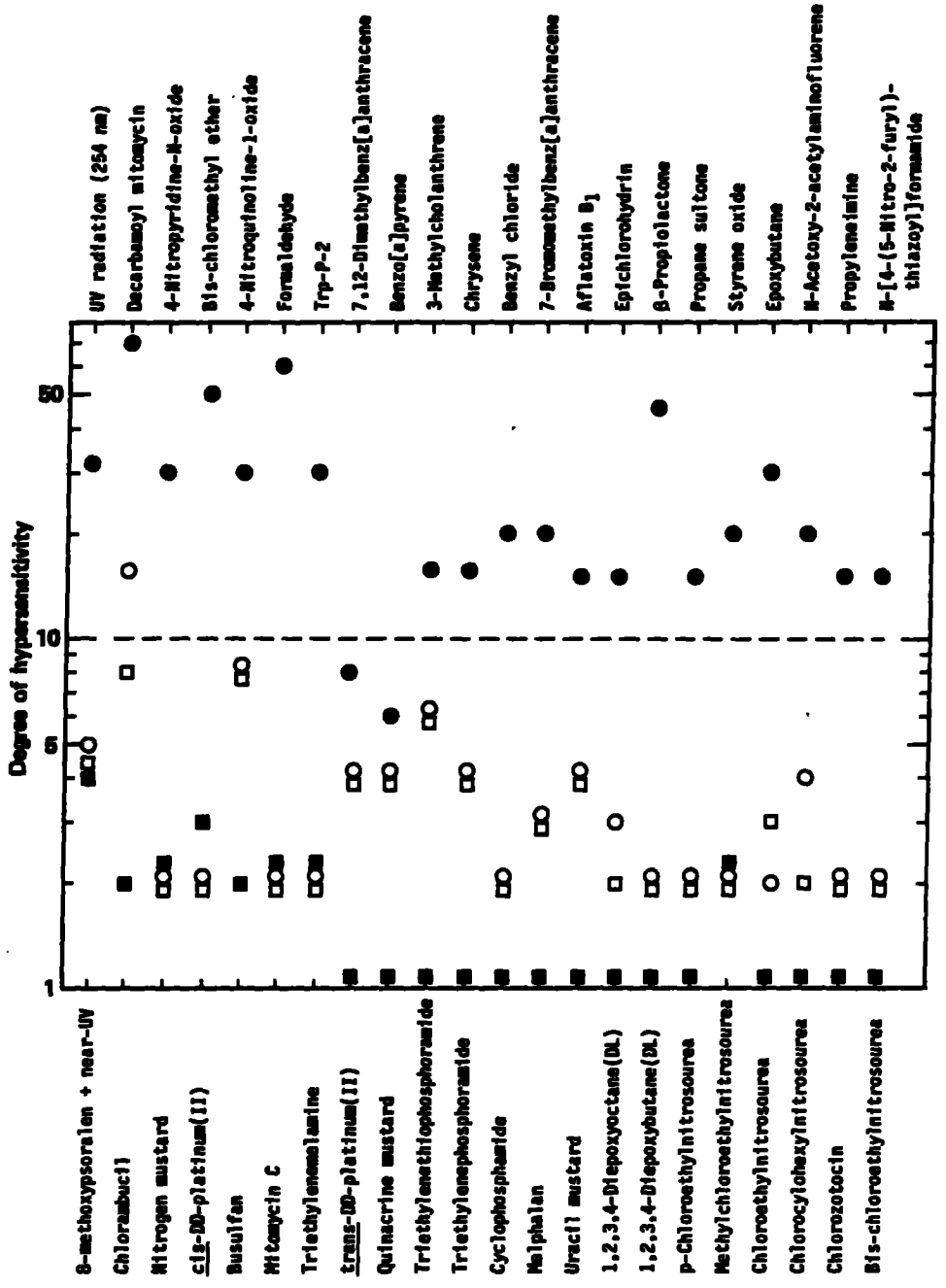


Figure 1. Degree of hypersensitivity of mutants to killing by bulky mono- and bifunctional agents. Symbols: UV5 (□) and UV4 (○) for monofunctional agents, listed at the top; UV5 (■) and UV4 (or UV20) (●) for bifunctional agents at the bottom. Data redrawn from Hoy et al., 1984, 1985.

bifunctional compounds defines two discrete subgroups (Hoy et al., 1985). These relationships are illustrated by the results summarized in Figure 1 for 22 monofunctional agents and 22 cross-linking agents that were tested in a rapid differential cytotoxicity assay (Hoy et al., 1984, 1985). On this graph a value of 1 means that a mutant has the same sensitivity to killing as normal cells. Both mutants UV5 and UV4, which belong to Complementation Groups 1 and 2, respectively, have moderate hypersensitivity (2-8 fold) to monofunctional compounds. In contrast, only UV4 has extreme hypersensitivity (>10-fold) to the bifunctional compounds; UV5 is either slightly hypersensitive or normal in its response. For two of the bifunctional compounds (quinacrine mustard and trans-DD-platinum(II)), the response of UV4 was less than 10-fold. As discussed by Hoy et al.(1985), this lesser sensitivity may be accounted for by the presence of other classes of lesions besides DNA interstrand cross-links that contribute to toxicity. Examination of mutants in the other complementation groups, and of independent mutants within groups, indicates that Groups 2 and 4 are characteristically very sensitive to cross-linkers while Groups 1, 3, and 5 are not. The biochemical basis of this differential sensitivity is not known.

EM9, A Mutant With Very High Sister-Chromatid Exchange

CHO mutant line EM9 was isolated on the basis of hypersensitivity to killing by ethyl methanesulfonate (Thompson et al., 1980) and was found to be deficient in repairing DNA strand breaks after exposure to ionizing radiation or ethyl methanesulfonate (Thompson et al., 1982b). The profile of hypersensitivity of EM9 to other mutagens is narrow. EM9 has marked sensitivity to alkyl methanesulfonates and alkyl sulfates but only slight sensitivity to nitrosoureas and nitrosoguanidines (L. Thompson and C. Hoy, unpublished results). It has normal sensitivity to DNA cross-linking agents and many compounds that form bulky monoadducts (Hoy et al., 1984, 1985). The nucleotide excision repair system in EM9 is functional (Thompson et al. 1984), and the specific biochemical defect has not been determined. DNA ligases (Chan et al., 1984) AP endonucleases (La Belle et al., 1984), and poly(ADP-ribose) metabolism (Ikejima et al., 1984) all appear to be normal in EM9. Perhaps the defect lies in

some factor that affects the structure of chromatin rather than an enzymatic activity per se.

EM9 cells share with cells from Bloom's syndrome the interesting property of a greatly elevated baseline frequency of sister-chromatid exchange (SCE), about 10 times the normal level (Thompson et al., 1982b). By using a monoclonal antibody to BrdUrd-containing DNA, the enhanced SCEs in EM9 were found to be attributable to the BrdUrd itself, which is routinely used in visualizing SCE (Pinkel et al., 1985).

Normal human cells contain a gene that corrects the defect in EM9. Tests for genetic complementation of EM9 were done using CldUrd as a selective agent since EM9 was found to be efficiently killed by this analog (Dillehay et al., 1984). Complementation was shown both by fusing cells to form hybrids and by transfecting with genomic DNA (Thompson et al., 1985c). The presence of the human gene resulted in normalization of SCE frequencies. Efforts are underway to isolate this human gene.

Mapping of DNA Repair Genes Using Hamster/Human Hybrids

The large number of complementation groups of XP mutations (9 at present for excision repair; Fischer et al., 1985) has raised the question whether these groups all correspond to single gene loci (Lambert and Lambert, 1985). The same issue applies to the 5 groups of UV-sensitive CHO mutants. One approach toward answering this question is to localize in the human karyotype the genes that complement the defects in the CHO mutants. Thus, by making CHO/human cell hybrids under the appropriate selective conditions, we have begun to map each of the complementing human genes. The mutation in strain UV20 (Group 2) was complemented by human chromosome 19 (Thompson et al., 1985b). Confirming evidence was reported by Rubin et al., 1985). Preliminary evidence suggests that UV5 may also be complemented by human chromosome 19 while each of the other 3 groups involves a different human chromosome (L. Thompson and M. Siciliano, unpublished results). Somewhat surprisingly, mutant EM9 is also complemented by a gene on chromosome 19 (M. Siciliano, A. Carrano, L. Thompson, in preparation).

The finding that several DNA repair genes are located

on one small human chromosome might be explained, at least partially, by the fact that a linkage group on this chromosome (including markers PEP-D and GPI) appears conserved between human and hamster and by the fact that CHO cells are monosomic for these loci (Siciliano et al., 1983). Thus, CHO mutations recovered preferentially from monosomic chromosomal regions could thereby lead to apparent clustering of human repair genes. However, the question remains as to whether the linkage of two genes for the incision step of nucleotide excision repair may have functional significance.

ACKNOWLEDGMENTS

I thank Kerry Brookman, Eddie Salazar, and Sheri Stewart for their valuable assistance in these studies, and A. Carrano and J. Fuscoe for reading the manuscript. This work was performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under Contract W-7405-ENG-48.

REFERENCES

Busch DB, Cleaver JE, Glaser DA (1980). Large-scale isolation of UV-sensitive clones of CHO cells. Somat Cell Genet 6: 407-418.

Chan JYH, Thompson LH, Becker FF (1984). DNA ligase activities appear normal in the CHO mutant EM9. Mutat Res 131: 209-214.

Cleaver JE (1983). Xeroderma pigmentosum. In Stanbury JB, Wyngaarden JB, Fredrickson DS, Goldstein JL, Brown MS (eds): "The Metabolic Basis of Inherited Disease", 5th edition, New York: McGraw-Hill, pp. 1227-1248.

Dillehay LE, Thompson LH, Carrano AV (1984). DNA strand breaks associated with halogenated pyrimidine incorporation. Mutat Res 131: 129-136.

Fischer E, Keijzer W, Thielmann HW, Popanda O, Bohnert E, Edler L, Jung EG, Bootsma D (1985). A ninth complementation group in xeroderma pigmentosum. Mutat Res 145: 217-225.

Hori T, Shiomi T, Sato K (1983). Human chromosome 13 compensates a DNA repair defect in UV-sensitive mouse cells by mouse-human cell hybridization. Proc Natl Acad Sci USA 80: 5655-5659.

Hoy CA, Salazar EP, Thompson LH (1984). Rapid detection of DNA-damaging agents using repair-deficient CHO cells. Mutat Res 130: 321-332.

Hoy CA, Thompson LH, Mooney CA, Salazar EP (1985). Defective DNA cross-link removal in Chinese hamster cell mutants hypersensitive to bifunctional alkylating agents. Cancer Res 45: 1737-1743.

Ikejima M, Bohannon D, Gill DM, Thompson LH (1984). Poly(ADP-ribose) metabolism appears normal in EM9, a mutagen-sensitive mutant of CHO cells. Mutat Res 128: 213-220.

La Belle M, Linn S, Thompson LH (1984). Apurinic/aprimidinic endonuclease activities appear normal in the CHO-cell ethyl methanesulfonate-sensitive mutant, EM9. Mutat Res 141: 41-44.

Lambert WC, Lambert MW, (1985). Co-recessive inheritance: A model for DNA repair, genetic disease and carcinogenesis. Mutat Res 145: 227-234.

Pinkel D, Thompson LH, Gray JW, Vanderlaan M (1985). Measurement of sister chromatid exchanges at very low BrdUrd substitution levels using a monoclonal antibody. Cancer Res: submitted.

Rubin JS, Joyner AL, Bernstein A, Whitmore GF (1983). Molecular identification of a human DNA repair gene following DNA-mediated gene transfer. Nature 306: 206-208.

Rubin JS, Prideaux VR, Willard HF, Dulhanty AM, Whitmore GF, Bernstein F (1985). Molecular cloning and chromosomal localization of DNA sequences associated with a human DNA repair gene. Mol Cell Biol 5: 398-405.

Sato K, Hieda N (1979). Isolation of a mammalian cell mutant sensitive to 4-nitroquinoline-1-oxide. Int J Radiat Biol 35: 83-87.

Siciliano MJ, Stallings RL, Adair GM, Humphreys RM, Siciliano J (1983). Provisional assignment of TPI, GPI, and PEPD to Chinese hamster autosomes 8 and 9: a cytogenetic basis for functional haploidy of an autosomal linkage group in CHO cells. Cytogenet Cell Genet 35: 15-20.

Stamato TD, Waldren CA (1977). Isolation of UV-sensitive variants of CHO-K1 by nylon cloth replica plating. Somat Cell Genet 3: 431-440.

Thompson LH, Carrano AV (1983). Analysis of mammalian cell mutagenesis and DNA repair using in vitro selected CHO mutants. In Friedberg EC, Bridges BR (eds): "Cellular

Responses to DNA Damage," New York: Alan R. Liss, pp. 125-143.

Thompson LH, Rubin JS, Cleaver JE, Whitmore GF, Brookman K (1980). A screening method for isolating DNA repair-deficient mutants of CHO cells. Somat Cell Genet 6: 391-405.

Thompson LH, Busch DB, Brookman K, Mooney CL, Glaser DA (1981). Genetic diversity of UV-sensitive DNA repair mutants of Chinese hamster ovary cells. Proc Natl Acad Sci USA 78: 3734-3737.

Thompson LH, Brookman KW, Dillehay LE, Mooney CL, Carrano AV (1982a). Hypersensitivity to mutation and sister-chromatid-exchange induction in CHO cell mutants defective in incising DNA containing UV lesions. Somat Cell Genet 8: 759-773.

Thompson LH, Brookman KW, Dillehay LE, Carrano AV, Mazrimas JA, Mooney CL, Minkler JL (1982b). A CHO-cell strain having hypersensitivity to mutagens, a defect in DNA strand-break repair, and an extraordinary baseline frequency of sister chromatid exchange. Mutat Res 95: 427-440.

Thompson LH, Brookman KW, Carrano AV, Dillehay LE (1982c). Role of DNA repair in mutagenesis of Chinese hamster ovary cells by 7-bromomethylbenz[a]anthracene. Proc Natl Acad Sci USA 79: 534-538.

Thompson LH, Salazar EP, Brookman KW, Hoy CA (1983a). Hypersensitivity to cell killing and mutation induction by chemical carcinogens in an excision-repair-deficient mutant of CHO cells. Mutat Res 112: 329-344.

Thompson LH, Carrano AV, Salazar E, Felton JS, Hatch FT (1983b). Comparative genotoxic effects of the cooked-food-related mutagens Trp-P-2 and IQ in bacteria and cultured mammalian cells. Mutat Res 117: 243-257.

Thompson LH, Brookman KB, Mooney CL (1984). Repair of DNA adducts in asynchronous CHO cells and the role of repair in cell killing and mutation induction in synchronous cells treated with 7-bromomethylbenz[a]anthracene. Somat Cell Mol Genet 10: 183-194.

Thompson LH, Mooney C, Brookman K (1985a). Genetic complementation between UV-sensitive CHO mutants and xeroderma pigmentosum fibroblasts. Mutat Res 150: in press.

Thompson LH, Mooney CL, Burkhart-Schultz K, Carrano AV, Siciliano MJ (1985b). Correction of a nucleotide-excision-repair mutation by human chromosome 19 in hamster-human hybrid cells. Somat Cell Mol

Genet 11: 87-92.

Thompson LH, Brookman KW, Minkler JL, Fuscoe JC, Henning KA, Carrano AV (1985c). DNA-mediated transfer of a human DNA repair gene that controls sister chromatid exchange. Mol Cell Biol 5: 881-884.

Walker GC, Marsh L, Dodson, LA (1985). Genetic analysis of DNA repair: inference and extrapolation. Ann Rev Genet. in press.

Westerveld A, Hoeijmakers JHJ, van Duin M, de Wit J, Odijk H, Pastink A, Wood RD, Bootsma D (1984). Molecular cloning of a human DNA repair gene. Nature 310: 425-429.

Wood RD, Burki, HJ (1982). Repair capability and the cellular age response for killing and mutation induction after UV. Mutat Res 95: 505-514.